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REMARKS

Claims 39-43, and 46-68 constitute the pending claims in the present application. Applicants respectfully request reconsideration in view of the following remarks. Issues raised by the Examiner will be addressed below in the order they appear in the prior Office Action.

Applicants have amended claims 39-41 by specifying that the composition in each claim is administered at a sub-immunosuppressive amount. Applicants have provided this amendment solely to expedite prosecution. Applicants reserve the right to pursue claims of similar or differing scope as the unamended claims at a later time. Issues raised by the Examiner will be addressed below in the order they appear in the prior Office Action. Applicants respectfully request reconsideration in view of the following remarks.

1. The disclosure was objected to, and requested the claim for priority inserted by the amendment filed July 24, 2002 be deleted. Applicants have herein made the requested amendments to the specification.

2. Claim 42 is rejected under 35 U.S.C. 112, 1st paragraph. The Office Action alleges that there is no original disclosure of the cause of the glucose intolerance recited in claim 42. The Office Action also stated that the Examiner was unable to obtain a copy of the cited article. Applicants respectfully traverse this rejection.

For the Examiner's convenience, Applicants have attached with the present response a copy of the Gallwitz et al. article and the translation thereof (Exhibit A), which discloses the use of male and female GLP-1 "knockout" mice (GLP-$1R^{-/-}$) to demonstrate the importance of GLP-1 for the regulation of the blood sugar level, wherein a pathological glucose tolerance with elevated blood glucose levels was found in the mice postprandially up to two hours after the beginning of the test in the oral glucose tolerance test (See Exhibit A Gallwitz et al. translation page 2, lines 12-18). Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection.

3. Claims 53, 65, and 66 are objected to for certain informalities. Applicants have amended the claims to correct these informalities as follows:

a. Applicants have amended the claim dependencies of claim 53 to limit the dependency to claim 41. Applicants assert that this amendment in no way narrows the scope of the claim because the amendment simply corrects the presence of a redundant limitation. Specifically, since claims 38-40 already contain the limitation that the composition be administered orally, it would be redundant to have claim 53, a claim directed to oral administration, depend from these claims.

b. Applicants have inserted an "or" in claim 65 (page 15, line 13 in the previously filed Office Action response) before the last chemical structure in the line.

c. Applicants have deleted the definition of R6 in its entirety from claim 66.

In light of the foregoing amendments, Applicants respectfully request reconsideration and withdrawal of the objections.

4. Claims 38-42 and 46-48 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-14 and 16-40 of copending Application No. 09/601,432.

Applicants will address this rejection when the rejection is no longer provisional.

5. Claims 38-42 and 46-48 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 38-132 of copending Application No. 10/190,267.

Applicants will address this rejection when the rejection is no longer provisional.

6. Applicants acknowledge that the effective filing date of the instant claims 38-41 and 46-48 is deemed to be February 2, 1998, the filing date of provisional application 60/073,409. Applicants further favorably acknowledge that Deacon et al. (Diabetes, Vol. 47, pages 764-769) and WO Patent Application 98/25644 are not available as prior art against these claims. Applicants note with appreciation that Drucker, U.S. Pat. No. 5,952,301, is no longer applied against the instant claims because Drucker does not contain any disclosure concerning the use of dipeptidylpeptidase inhibitors.

7. Claims 38, 39, 41, 44-63, 65, 66, and 68 are rejected under 35 U.S.C. 103(a) as being obvious over the Balkan et al. abstract, in view of the WO Patent Application 93/08259 and further in view of Efendic et al. (U.S. Patent No. 5,631,224). Applicants respectfully traverse this rejection to the extent it is maintained over the claims as amended.

Claims 38, 39, and 41 are the independent claims in the rejected set. Applicants have cancelled claims 44 and 45. Claim 38 is directed to a method for modifying glucose metabolism **in a glucose intolerant animal**, comprising administering to the animal, in a single daily oral dosage, a composition including one or more protease inhibitors which inhibit DPIV-mediated proteolysis with a Ki of less than about 10 nM in an amount sufficient to modify glucose metabolism but not sufficient to suppress the immune system of the animal. Claim 39 is directed to a method for modifying glucose metabolism **in a glucose intolerant animal**, comprising administering to the animal, in a single daily oral dosage, a composition including one or more protease inhibitors which inhibit the proteolysis of glucagon-like peptide 1 (GLP-1) with a Ki of less than about 10 nM in an amount sufficient to modify glucose metabolism but not sufficient to suppress the immune system of the animal. Finally, claim 41 is directed to a method for modifying glucose metabolism **of a glucose intolerant animal**, comprising administering to the animal a composition including a boronyl peptidomimetic inhibitor of a peptide selected from Pro-Pro, Ala-Pro, and (D)-Ala-(L)-Ala. In each case the composition is administered at a sub-immunosuppressive amount. Applicants assert that the cited references, alone or in combination, do not render these claims obvious.

Applicants point out that Balkan et al. is unavailable as prior art under 35 U.S.C. § 102(a) as set forth in the declaration under 37 C.F.R. § 1.131 filed herewith (Exhibit B). Because the claimed invention was made prior to the publication of Balkan et al., that reference is unavailable as prior art against the pending claims.

Additionally, Applicants respectfully point out that Balkan et al., either alone or in combination with the rest of the cited references, does not teach or suggest all the elements of the instant claims. Specifically, Balkan et al. does not teach administering a DPIV inhibitor to a glucose intolerant animal at sub-immunosuppresive amounts that are nevertheless sufficient to treat Type II diabetes.

Applicants further point out that Balkan et al. suffers from multiple deficiencies including (a) the one and only inhibitor disclosed therein is a proprietary compound whose structure was not available to a skilled person (contrary to the Office Action's assertion that the compound used in the reference is valine pyrrolidide, Balkan et al. does not identify the compound as valine pyrrolidide); (b) if the compound is indeed valine pyrrolidide, it does not render claim 41 obvious because the claim is directed to boronyl pepetidomimetics of Pro-Pro, Ala-Pro, and (D)-Ala-(L)-Ala; and (c) the compound used by Balkan et al. was not administered orally. Thus, Applicants respectfully submit that Balkan et al. does not provide the requisite motivation for one of ordinary skill in the art to orally administer the DPIV inhibitor compounds of the instant claims to modify glucose metabolism in a glucose intolerant animal with a single daily dosage. Similarly, Balkan et al. does not provide motivation for one of skill in the art to administer a composition comprising a boronyl peptidomimetic inhibitor to modify glucose metabolism of a glucose intolerant animal.

The '259 application does not compensate for the deficiencies in Balkan et al., and moreover Applicants assert that there is no motivation to combine these two references. The '259 application does not teach or suggest administering DPIV inhibitors to glucose intolerant animals at sub-immunosuppressive amounts. Moreover, even under the assumption that the compound used in Balkan et al. is valine pyrrolidide, this compound is structurally very different than any of the compounds disclosed in the '259 application. Furthermore, the reported K_is of the compounds in the '309 application are orders of magnitude different than the K_i of Balkan's compounds. There is simply no teaching or suggestion found in either of the references or in the combinations thereof that would give one of ordinary skill in the art the requisite reasonable expectation of success that that the compounds of the '259 application would work in the same manner as Balkan's compound.

Additionally, the '259 application does not teach or suggest a method for modifying glucose metabolism in a glucose intolerant animal with a single daily oral dosage of a DP IV inhibitor, or for modifying glucose metabolism of a glucose intolerant animal, by administering to the animal a composition including a boronyl peptidomimetic inhibitor. Contrary to the Office Action's assertions, the '259 application does not give an enabling disclosure that teaches administering the compounds disclosed therein to a glucose intolerant animal, let alone an animal

with Type II diabetes, in the form of a single daily oral formulation. Thus, even if the teachings of Balkan and the '259 patent were to be combined, one of ordinary skill in the art at best would have only had a motivation to try the compounds of the '259 application. But this motivation to try is not sufficient to establish a prima facie case of obviousness because it exists in the absence of any reasonable expectation of success that such an endeavor would result in modifying glucose metabolism in a glucose intolerant animal with after administration of a single daily oral dosage of a DP IV inhibitor.

The third reference cited by the Office Action is Efendic et al. (U.S. Patent No. 5,631,224). Applicants assert that this reference is not relevant to any of the rejected claims because the reference is directed to administration of various analogs of the GLP-1 protein in conjunction with oral hypoglycemic agents. Applicants point out that none of the rejected claims relate to administration of GLP-1 to a patient. Moreover, the oral hypoglycemic agents bear no structural or functional resemblance to the compounds administered in the present claims. Applicants assert that Efendic et al., alone or in combination with Balkan et al. and the '259 application, has little to no relevance to the (non)obviousness of the instant claims. Furthermore, combining Efendic et al. with Balkan et al. or the '259 application does not strengthen the argument. Therefore, Applicants assert that Efendic et al., alone or in combination with the other references, does not provide the requisite motivation or reasonable expectation of success to render claims 38, 39, 41, or claims dependent thereon obvious.

In light of the arguments presented above, Applicants respectfully request reconsideration and removal of the rejection.

10. Claims 38-40, 46-53, and 68 are rejected under 35 U.S.C. 102(e) as being anticipated by Villhauer (U.S. Patent No. 6,011,055). Applicants respectfully traverse the rejection to the extent it is maintained over the claims as amended.

Applicants had argued in their earlier response that Villhauer does not anticipate the present claims because it does not provide an enabling disclosure for treating glucose intolerant animals with DPIV inhibitors. Notwithstanding these arguments however, the present Office Action maintained the rejection on grounds that a U.S. patent is presumed operable and enabled, and cited to MPEP 716.07 and 2121 for support for the notion that the burden is on Applicants to

provide evidence of inoperability or lack of enablement. Applicants assert that the Office Action has misapplied the MPEP.

In order for Villhauer to anticipate claims 38-40, it must teach in an enabling manner the method of modifying glucose metabolism of a glucose intolerant animal with a single daily oral dosage of a DPIV inhibitor. Applicants reiterate that Villhauer does not provide an enabling disclosure for modifying glucose metabolism of a glucose intolerant animal. Applicants point out that for a prior art to be anticipatory it has to have an enabling disclosure (see MPEP 2131.01). While the Office Action is correct that the MPEP states in 716.07 that a U.S. patent is presumed enabled, it is the claims of the U.S. patent that are presumed enabled. It would be absurd to interpret that section to mean that everything, including unsupported prophetic utterances, disclosed in a U.S. patent are enabled. Applicants point out that Villhauer does not contain a single claim directed to a method for modifying glucose metabolism in a glucose intolerant animal. Thus, just because Villhauer mentions diabetes melitus and impaired glucose tolerance in the Abstract, Applicants submit that this does not constitute an enabled disclosure. As such, citation to MPEP 716.07 is moot.

With respect to MPEP 2121, the relevant quote from that section is as follows:

> "When the reference relied on expressly anticipates or makes obvious all of the elements of the claimed invention, the reference is presumed to be operable. Once such a reference is found, the burden is on applicant to provide facts rebutting the presumption of operability. *In re Sasse*, 629 F.2d 675, 207 USPQ 107 (CCPA 1980)."

Even the Office Action admits that the Villhauer reference does not expressly anticipate the instant claims because the Office Action is pursuing the rejection under inherency. Applicants reiterate that since Villhauer does not expressly teach modifying glucose metabolism of a glucose intolerant animal, the citation to MPEP 2121 is also moot.

What is left is Applicant's original contention, which is squarely based upon proper PTO practice under MPEP 2131.01. Since Villhauer does not have a single claim or working example directed to modifying glucose metabolism of a glucose intolerant animal, it would be improper to accord Villhauer a presumption of enablement for the purpose of anticipating the instant claims.

If more direct proof of non-enablement is needed, one only needs to note that the only in vivo experiment Villhauer provides is the administration of compounds to normal male Sprague-Dawley rats and measurement of early insulin response (see col. 9, line 66 to col. 10, line 28). There is no indication that these rats are glucose intolerant, have diabetes, or are non-insulin dependent diabetics. In contrast, Applicants show in Figure 4 that a GLP-1 receptor -/- transgenic mice has high blood glucose which is ameliorated by administration of a compound of the present invention. The animal models used by Applicants are truly glucose intolerant. As such, Applicants assert that Villhauer does not teach all the elements of the instant claims, and therefore fails to anticipate the claims.

11. Claims 38-41, 44-52, 54-57, 59, 60, 63 and 66 are rejected under 35 U.S.C. 102(b) as being anticipated by inventor Demuth's German Patent 19616486. Applicants traverse the rejection to the extent that it is maintained over the claims as amended.

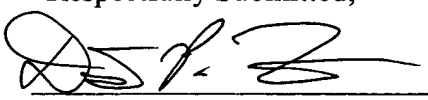
Applicants point out that, as the Examiner has already acknowledged, Demuth was published less than one year prior to the priority date of the instant application (October 30, 1997), therefore only a rejection under 102(a) can be applied.

Furthermore, Applicants assert that Demuth et al. is unavailable as prior art under 35 U.S.C. § 102(a) as set forth in the declaration under 37 C.F.R. § 1.131 filed herewith (Exhibit B). As the declaration states, the invention had been conceived prior to June 1997 and reduced to practice within four months. Since the invention had been reduced to practice prior to October 1997, Demuth is unavailable as prior art against the pending claims. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants submit that the pending claims are in condition for allowance. Early and favorable reconsideration is respectfully solicited. The Examiner may address any questions raised by this submission to the undersigned at 617-951-7000. Should an extension of time be required, Applicants hereby petition for same and request that the extension fee and any other fee required for timely consideration of this submission be charged to **Deposit Account No. 18-1945.**

Respectfully Submitted,



David P. Halstead, Ph.D.
Reg. No. 44,735

Date: April 20, 2004

Customer No: 28120
Docketing Specialist
Ropes & Gray LLP
One International Place
Boston, MA 02110
Phone: 617-951-7000
Fax: 617-951-7050

COMMENTATED REVIEW

GLP-1 RECEPTOR GENE "KNOCKOUT" CAUSES GLUCOSE INTOLERANCE, BUT NO DISRUPTION OF EATING BEHAVIOR

GALLWITZ, B., AND SCHMIDT. W.E.

SOURCE: Z. Gastroenterol. 1997; 35: 655-658

Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene

Scrocchi, LA, Brown, TJ, MacLusky, N, Brubaker, PL, Auerbach, AB, Joyner, AL, Drucker, DJ.

Nature Medicine 1996; 2: 1254-8

SUMMARY

The gastrointestinal hormone glucagon-like peptide-1 (GLP-1) stimulates postprandial insulin secretion (*Kreymann B* et al. Lancet 1987; ii: 1300-4). In patients with type II diabetes mellitus, exogenous doses of GLP-1 improve postprandial glucose metabolism (*Nauch MA* et al. Diabetologia 1993; 36: 741-4). Further, according to more recent studies, GLP-1 appears to play an important role as a central mediator for satiety (*Turton MD* et al. Nature 1996; 379: 69-72). However, since all other peptides influence insulin secretion and satiety behavior, the relative importance of GLP-1 as a physiological stimulator of insulin secretion and as a central mediator of feeding behavior was investigated by the research group of *Drucker* in the present study on a mouse model with a missing GLP-1 receptor.

Mice were first bred with the targeted exclusion of the GLP-1 receptor gene (GLP-1 receptor "knockout" GLP-1R$^{-/-}$). For this purpose, a targeting vector was constructed, which replaces different exons of the GLP-1 receptor. The genomic DNA of the transgenic animals showed a complete exclusion of the GLP-1 receptor gene. The

recombinant GLP-1R$^{-/-}$ allele obtained in this way was reproduced with the expected frequency according to Mendel's law in the next generation, and thus GLP-1$^{-/-}$ mutants could be bred. These mice are viable, develop normally, and phenotypically appear to be completely unremarkable. mRNA transcripts of the GLP-1 receptor were not found in the pancreas, hypothalamus and lungs, where the GLP-1 receptor is expressed to a great extent in normal animals. The histological structure of these organs was completely unremarkable.

Address of the commentators: Prof. B. Gallwitz, M.D., W. E. Schmidt, M.D., First Medical Clinic of the Christian Albrecht University of Kiel, Schittenhelmstr. 12, 24105 Kiel

Oral glucose tolerance tests were conducted in male and female GLP-1R$^{-/-}$ mice in order to investigate the importance of GLP-1 for the regulation of the blood sugar level. A pathological glucose tolerance with elevated blood glucose levels was found in these mice postprandially up to two hours after the beginning of the test in the oral glucose tolerance test. The fasting glucose levels were also elevated in most of the mice. The disturbance in glucose tolerance was more pronounced in the male animals than in the females.

When serum insulin concentrations were measured simultaneously, the GLP-1R$^{-/-}$ mice showed comparable fasting values when compared with the control animals. Thirty minutes after oral ingestion of glucose, the serum insulin concentration, however, was reduced by approximately one-third in the knockout animals. The values after one hour were again equal in the GLP-1R$^{-/-}$ mice and the controls. The parallelly measured glucagon levels were the same in both groups of animals during the oral glucose tolerance test.

In order to show that GLP-1 decisively participates in the regulation of the blood glucose concentration, glucose was also administered intraperitoneally to normal mice and GLP-1R$^{-/-}$ mice. With this route of glucose introduction, the transgenic animals also showed a pathological glucose tolerance. In the male animals, the glucose intolerance was more pronounced than in the oral glucose tolerance test.

As a control test to determine that the biological effects of GLP-1 are not mediated by other receptors, a third test series was conducted in which oral glucose tolerance tests were carried out with the simultaneous administration of pharmacological doses of 100 µg of GLP-1. In this case, the elevation of blood sugar could be suppressed in the normal control animals by exogenous GLP-1 administration in the oral glucose tolerance test. The administration of GLP-1 had no effect on blood sugar in the GLP-1R$^{-/-}$ mice.

Since the research team of *Bloom* (*Turton MD* et al. Nature 1996; 379: 69-72) had recently postulated that GLP-1 is an important regulator of satiety in the CNS, the eating behavior of GLP-1R$^{-/-}$ mice was investigated under various conditions. Over a time period of 24 weeks, the body weight in these animals was identical to that of normal controls, which indicated that under normal conditions, GLP-1 certainly does not have a dominant role in the regulation of body weight. In the case of fasting animals, no difference was found in the quantity of food ingested. Thus, the absence of the GLP-1 receptor leads neither to a change in the body weight nor to a change in eating behavior. Intracerebroventricular (icv) administration of 5 µg of GLP-1, which prevents the ingestion of feed in normal animals, had no effect in the case of GLP-1R$^{-/-}$ mice. Autoradiographs with ^{125}I-GLP-1 showed that the transgenic mice do not express GLP-1 receptors. Thus it was shown that pancreatic and central GLP-1 receptors are structurally identical and are coded by the same gene.

From their experiments, the authors conclude that GLP-1 is a physiologically important endocrine hormone and thus decisively contributes to postprandial stimulation of insulin secretion. From tests in which glucose was given intraperitoneally, it results that GLP-1, independently of the route of introduction, decisively regulates glucose homeostasis, possibly by stimulating peripheral glucose utilization. The role of GLP-1 as a central regulator of satiety appears to be less important than the peripheral effect of GLP-1 as a stimulator of insulin secretion. In the opinion of the authors, the experiments underscore the importance of GLP-1 as a physiological regulator of the blood sugar level. They therefore see possibilities of utilizing GLP-1 therapeutically in future in the treatment of diabetes.

COMMENTARY

In the beginning of the 90's, due to new molecular genetic methods, it was possible to breed animal models with specifically excluded genes (*Tybulewicz V* et al. Cell 1991; 65: 1153-63; *Nagy A* et al. Proc Natl Acad Sci USA 1993; 90: 8424-8; *Nagy A, Rossant J.* In: Gene targeting: A practical approach, *Joyner AL* (ed.). Oxford, Oxford Univ Press 1993; 147-78; *Wurst W, Joyner AL.* In: Gene targeting: A practical approach. *Joyner AL* (eds.). Oxford, Oxford Univ Press 1993; 33-61). These so-called "knockout" mutants have since then become important *in-vivo* models for investigation in order to examine the consequences of the absence of a protein on the organism. Since highly specific ligands with exclusive receptor antagonistic activity are still not available for many hormone receptors, animals are increasingly bred with targeted excluded receptor genes. Several "knockout" constructs, however, lead to lethal mutations, so that this technique is limited. In the present study, the research group of Drucker has been successful for the first time in breeding a mice mutant which does not possess a pancreatic GLP-1 receptor. They could further show that this type of GLP-1 receptor mediates all the important biological functions of GLP-1 peripherally and in the CNS. This mouse mutant not only has an unremarkable phenotype, but it also develops normally and is fully viable. It is thus excellently suitable as an animal model for a complete GLP-1 resistence, which is brought about by an absence of the GLP-1 receptor. Up until now, only the exendin fragment exendin (9-39) (*Göke R* et al. J Biol Chem 1993; 268: 19650-5; *Thorens B* et al. Diabetes 1993; 42: 1678-82; *Kolligs F* et al. Diabetes 1995; 44: 16-9) could be used as a GLP-1 receptor antagonist, but this also acts as an antagonist to the receptor for gastric inhibitory polypeptide (GIP) (*Gremlich S* et al. Diabetes 1995; 44: 1202-8). Since GIP also stimulates postprandial insulin secretion (*Creutzfeldt W.* Diabetologia 1979; 16: 75-85; *Creutzfeldt W, Ebert R.* Diabetologia 1985; 28: 565-73; *Creutzfeldt W, Ebert R.* In: The exocrine pancreas: Biology, pathobiology and disease. *Go VI* et al. (eds). New York, Raven Press 1986; 333-46), no exclusive information can be obtained on the effect of GLP-1 by experiments with exendin (9-39) *in vivo.*

GLP-1 and its pronounced stimulation of insulin secretion have been known for approximately ten years (*Schmidt WE* et al. Diabetologia 1985; 28: 704-7; *Mojsov S* et al.

J Clin Invest 1987: 79: 619-9; *Kreymann B* et al. Lancet 1987; ii: 1300-4). Of all gastrointestinal hormones, GLP-1 is the most potent stimulator of insulin secretion and thus decisively contributes to the endocrine hormone effect (*Orskov C.* Diabetologia 1992; 35: 701-11). Therapeutic possibilities for GLP-1 in type II diabetes mellitus have been intensively investigated for several years by various research teams (*Nathan DM* et al. Diabetes Care 1992; 15: 270-6; *Gutniak M* et al. N Engl J Med 1992; 326: 1316-22; *Nauck MA* et al. Diabetologia 1993; 36: 741-4; *Holz GG* et al. Nature 1993; 361: 362-5; *Thorens B, Waeber G.* Diabetes 1993; 42: 1219-25; *Deacon CF* et al. Diabetes 1995; 44: 1126-31; *Ritzel R* et al. Diabetologia 1995; 38: 720-5). The oral glucose tolerance tests conducted in the GLP-1R$^{-/-}$ mice impressively elucidate the effects that the absence of the GLP-1 response has on postprandial blood glucose and serum insulin levels in these animals. The only surprise in these results that were expected is that there is a pronounced difference in blood glucose between male and female GLP-1R$^{-/-}$ mice. A sex-specific different action of GLP-1 has not been previously described. In future clinical studies on patients with type II diabetes mellitus, therefore, this possible difference should be monitored. It must also be clarified in animal experiments how this sex-specific difference in the GLP-1 effect is mediated. The curves for insulin concentration in serum with maximal stimulation of insulin secretion in control animals 30 minutes after oral glucose loading, which have been presented by *Drucker* and colleagues, supports earlier data that GLP-1 primarily stimulates the first phase of insulin secretion (*Weir CG* et al. Diabetes 1989; 38: 338-42; *Orskov C* et al. Diabetes 1993; 42: 658-61). The lack of effect of exogenous GLP-1 doses in oral glucose tolerance tests supported the complete absence of the GLP-1 receptor or another receptor that could mediate the biological GLP-1 effect, in the transgenic animals. Unfortunately, intravenous administrations of glucose with the same blood glucose effect were not conducted in parallel on the same GLP-1R$^{-/-}$ mice and on the control animals, along with the oral glucose tolerance tests. The endocrine hormone effect and its GLP-1-mediated component could be precisely quantified by such experiments.

There are hints from the research team of *Ensinck* and colleagues that GLP-1 not only decreases postprandially the glucose concentration in plasma via a stimulation of insulin secretion, but also stimulates glucose utilization via insulin-independent

mechanisms (*D'Alessio DA* et al. J Clin Invest 1994; 93: 2263-6; *D'Alessio DA* et al. Diabetes 1995; 44: 1433-7; *D'Alessio DA* et al. J Clin Invest 1996; 97: 133-8). Glucagon secretion, e.g., is also inhibited by GLP-1, for which reason lower blood glucose levels result (*Nauck MA* et al. Diabetologia 1993; 36: 741-4; *Ritzel R* et al. Diabetologia 1995; 38: 720-5). *Drucker* and colleagues thus postulated that glucose utilization must be pathological in the GLP-1R$^{-/-}$ mice, independently of the route of glucose introduction, and observed the course of glucose concentration after intraperitoneal injection of glucose. The blood sugar was elevated for a longer period and was more pronounced in GLP-1R$^{-/-}$ mice than in normal animals also after intraperitoneal administration of glucose. The authors concluded that even when circumventing the insulin enteric pathway, the absent GLP-1 effect results in a pathological glucose utilization and thus insulin-independent influences of GLP-1 on glucose utilization must be important. However, this is only partially correct. Intraperitoneally applied glucose cannot in fact release GLP-1 directly in a physiological pathway via intraluminal stimulation mechanisms in the intestine, but it is not yet clear how carbohydrates stimulate the release of GLP-1. The GLP-1 release occurs primarily in the L cells of the distal part of the ileum (*Eissele R* et al. Eur J Clin Invest 1992; 22: 283-91), where oral glucose does not reach, since it is already resorbed in the proximal segments of the intestine. It has not yet been completely excluded that in addition to nerve stimulation by stretch stimuli in the proximal segments of the gastrointestinal tract, the glucose increase in the blood of the portal vein also leads to increased release of GLP-1. An increased glucose concentration also surges for a short time at least in the portal circulation due to the intraperitoneal administration of glucose, which corresponds to that of glucose given orally. Only the inhibiting effect of GLP-1 on the emptying of the stomach is avoided (*Willms B* et al. J Clin Endocrinol Metab 1996; 81: 327-32) in the case of intraperitoneal glucose administration when compared to oral administration. Whether or not extra-pancreatic effects of GLP-1 (e.g., stimulation of glucose uptake in skeletal muscle or fatty tissue, stimulation of glycogen synthesis in the liver) (*Egan JM* et al. Endocrinology 1994; 135: 2070-5; *Villanueva-Penacarrillo ML* et al. Diabetologia 1994; 37: 1163-6; *D'Alessio DA* et al. J Clin Invest 1994; 93: 2263-6; *D'Alessio DA* et al. Diabetes 1995; 44: 1433-7) play a role in glucose utilization can neither be clarified nor quantified with

the choice of intraperitoneal glucose administration selected by *Drucker* and colleagues. Investigations *in vitro* on the given tissues of mutant mice without the GLP-1 receptor and of normal controls would be necessary for this. Since the GLP-1R$^{-/-}$ mice can be easily reproduced, these investigations can be conducted in future without problem.

The recently published, sensational findings of the research team of *Bloom*, which grants to GLP-1 an important role as a central mediator of satiety (*Turton MD* et al. Nature 1996; 379: 69-72), are considerably weakened by the investigations of GLP-1R$^{-/-}$ mice. First of all, these mice also have a normal body weight over a long period of time, and, secondly, their eating behavior is unremarkable after a period of fasting. Icv administrations of GLP-1 were without effect in these animals, which demonstrates that the same "pancreatic" GLP-1 receptor is present in the CNS as in other organs. Autoradiographs with ^{125}I-labeled GLP-1 on brain sections of GLP-1R$^{-/-}$ mice showed that no other GLP-1 binding sites are additionally present in the CNS. It is obvious due to these findings that a regulation of eating behavior and of body weight in no way occurs primarily by central GLP-1. It has been known for a long time that many neuropeptides play a role in the central regulation of ingestion of food (*Morley JE* et al. Endocr Rev 1987; 8: 256-87; *Lee MC* et al. Neurosci Behav Rev 1994; 18: 313-23). Cholecystokinin (CCK) (*Moran TH* et al. Am J Physiol 1993; 265: G219-23) and bombesin participate in the mediation of satiety, while, on the other hand, neuropeptide Y (NPY) and galanin stimulate eating behavior (*Schick RR* et al. Brain Res 1991; 552: 232-9; *Schick RR* et al. Am J Physiol 1993: 264: R355-61). Up until now little has been known of the CNS interaction of these peptides. It could be pointed out for GLP-1, however, that it acts centrally via NPY-independent mechanisms (*Turton MD* et al. Nature 1996; 379: 69-72). Further, the product of the ob gene, leptin, appears to play an important role in the central regulation of satiety (*Pelleymounter MA* et al. Science 1995; 269: 540-3; *Halass JL* et al. Science 1995; 269: 543-6; *Campfield LA* et al. Science 1995; 269: 546-9). In mice with excluded NPY gene, which, like the GLP-1R$^{-/-}$ mice, were otherwise not adipose and showed normal eating behavior, an increased leptin sensitivity could be observed (*Erickson JC* et al. Nature 1996; 381: 415-8). The leptin sensitivity has still not been investigated for mice with excluded GLP-1 receptors. The certainly complex action and

interaction of GLP-1 with other neuromodulators in the CNS must be further clarified, and for this purpose, additional investigations in GLP-1R$^{-/-}$ mice would be meaningful.

Upshot

The present study supplies another piece of evidence for the importance of GLP-1 as an endocrine hormone. The pathological course of glucose concentration after oral or intraperitoneal administration of glucose in GLP-1R$^{-/-}$ mice underscores the influence of GLP-1 in the monitoring of glucose homeostasis and provides arguments for the fact that GLP-1 may have therapeutic potential for type II diabetes mellitus. In addition to these important results relative to the physiology of GLP-1, the present study again shows that "knockout" mice are useful experimental models, with a suitable experimental set-up, for investigating, in a targeted manner, the physiological effect of the absence of a protein.

GLP-1-Rezeptorgen »knockout« verursacht Glukoseintoleranz, aber keine Störung des Eßverhaltens

GALLWITZ, B., und SCHMIDT, W. E.

Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene

Scrocchi LA, Brown TJ, MacLusky N, Brubaker PL, Auerbach AB, Joyner AL, Drucker DJ.
Nature Medicine 1996; 2: 1254–8

ZUSAMMENFASSUNG

Das gastrointestinale Hormon Glucagon-like peptide-1 (GLP-1) stimuliert postprandial die Insulinsekretion (*Kreymann B* et al. Lancet 1987; ii: 1300–4). Bei Patienten mit Diabetes mellitus Typ II verbessern exogene GLP-1-Gaben den postprandialen Glukosestoffwechsel (*Nauck MA* et al. Diabetologia 1993; 36: 741–4). Ferner scheint GLP-1 nach neueren Arbeiten eine wichtige Rolle als zentraler Mediator der Sättigung zu spielen (*Turton MD* et al. Nature 1996; 379: 69–72). Da jedoch noch andere Peptide die Insulinsekretion und das Sättigungsverhalten beeinflussen, wurde von der Arbeitsgruppe von *Drucker* in der vorliegenden Arbeit an einem Mausmodell mit fehlendem GLP-1-Rezeptor die relative Wichtigkeit von GLP-1 als physiologischem Stimulator der Insulinsekretion und als zentralem Mediator des Freßverhaltens untersucht.

Zunächst wurden Mäuse mit einer gezielten Ausschaltung des GLP-1-Rezeptorgens (GLP-1-Rezeptor »knockout«, GLP-1R$^{-/-}$) gezüchtet. Hierzu wurde ein Targeting-Vektor konstruiert, der verschiedene Exons des GLP-1-Rezeptors ersetzt. Die genomische DNA der transgenen Tiere zeigte eine komplette Ausschaltung des GLP-1-Rezeptorgens. Das so erzielte rekombinante GLP-1R$^{-/-}$-Allel wurde mit der erwarteten Frequenz nach den Mendelschen Gesetzen an die nächste Generation weitergegeben, und es konnten so GLP-1$^{-/-}$-Mausmutanten gezüchtet werden. Diese Mäuse sind lebensfähig, entwickeln sich normal und erscheinen phänotypisch völlig unauffällig. mRNA-Transkripte des GLP-1-Rezeptors wurden in Pankreas, Hypothalamus und Lunge, wo bei normalen Tieren der GLP-1-Rezeptor in hohem Maße exprimiert wird, nicht gefunden. Die histologische Struktur dieser Organe war komplett unauffällig.

Um die Bedeutung von GLP-1 für die Regulation des Blutzuckerspiegels zu untersuchen, wurden bei männlichen und weiblichen GLP-1R$^{-/-}$-Mäusen orale Glukosetoleranztests durchgeführt. Bei diesen Mäusen fand sich im oralen Glukosetoleranztest eine pathologische Glukosetoleranz mit erhöhten Blutglukosewerten postprandial bis zwei Stunden nach Testbeginn. Auch die Nüchternglukosewerte waren bei den meisten Mäusen erhöht. Die Glukosetoleranzstörung war bei den männlichen Tieren ausgeprägter als bei den weiblichen.

Bei den gleichzeitig gemessenen Seruminsulinkonzentrationen zeigten die GLP-1R$^{-/-}$-Mäuse vergleichbare Nüchternwerte gegenüber Kontrolltieren. 30 Minuten nach oraler Aufnahme der Glukose war bei den »Knockout«-Tieren die Insulinkonzentration im Serum jedoch um etwa ein Drittel vermindert. Die Werte nach einer Stunde waren bei GLP-1R$^{-/-}$-Mäusen und Kontrolltieren wieder gleich. Die parallel gemessenen Glukagonspiegel waren bei beiden Tiergruppen während der oralen Glukosetoleranztests gleich.

Um zu zeigen, daß GLP-1 entscheidend an der Regulation der Blutglukosekonzentration beteiligt ist, wurde normalen Mäusen und GLP-1R$^{-/-}$-Mäusen Glukose auch intraperitoneal verabreicht. Auch bei dieser Glukosezufuhr zeigten die transgenen Tiere eine pathologische Glukosetoleranz. Bei den männlichen Tieren war die Glukoseintoleranz, wie schon im oralen Glukosetoleranztest, stärker ausgeprägt.

Als Kontrollversuch, daß biologische Effekte von GLP-1 nicht durch andere Rezeptoren vermittelt werden, diente eine dritte Versuchsreihe, in der orale Glukosetoleranztests mit gleichzeitiger Gabe pharmakologischer Dosen von 100 µg GLP-1 durchgeführt wurden. Hier ließ sich bei normalen Kontrolltieren durch exogene GLP-1-Gabe im oralen Glukosetoleranztest der Blutzuckeranstieg unterdrücken. Bei GLP-1R$^{-/-}$-Mäusen hatte die GLP-1-Gabe keinen Effekt auf den Blutzuckerverlauf.

Da die Arbeitsgruppe von *Bloom* (*Turton MD* et al. Nature 1996; 379: 69–72) kürzlich postuliert hatte, daß GLP-1 im ZNS ein wichtiger Regulator der Sätti-

Anschrift der Kommentatoren: Dr. med. B. Gallwitz, Prof. Dr. med. W. E. Schmidt, I. Medizinische Klinik der Christian-Albrechts-Universität Kiel, Schittenhelmstr. 12, 24105 Kiel

gung sei, wurde das Freßverhalten der GLP-1R$^{-/-}$-Mäuse unter verschiedenen Bedingungen untersucht. Das Körpergewicht war bei diesen Tieren über einen Zeitraum von 24 Wochen mit dem von normalen Kontrolltieren identisch, was darauf hinweist, daß unter normalen Bedingungen GLP-1 sicher keine dominierende Rolle bei der Regulation des Körpergewichtes hat. Bei nüchternen Tieren fand sich kein Unterschied in der Menge der aufgenommenen Nahrung. Das Fehlen des GLP-1-Rezeptors führt also weder zu einer Änderung des Körpergewichtes noch zu einer Änderung des Freßverhaltens. Intrazerebroventrikuläre (icv) Gaben von 5 µg GLP-1, die bei normalen Tieren die Futteraufnahme hemmen, hatten bei GLP-1R$^{-/-}$-Mäusen keinen Effekt. Autoradiographien mit ^{125}J-GLP-1 zeigten, daß die transgenen Mäuse keine GLP-1-Rezeptoren exprimieren. Damit wurde gezeigt, daß pankreatische und zentrale GLP-1-Rezeptoren strukturell identisch sind und durch dasselbe Gen kodiert werden.

Die Autoren folgern aus ihren Experimenten, daß GLP-1 ein physiologisch wichtiges Inkretin ist und somit entscheidend zur postprandialen Stimulation der Insulinsekretion beiträgt. Aus den Versuchen, bei denen Glukose intraperitoneal gegeben wurde, ergibt sich, daß GLP-1, unabhängig von der Route der Zufuhr, die Glukosehomöostase entscheidend reguliert, möglicherweise sogar durch eine Stimulierung der peripheren Glukoseverwertung. Die Rolle von GLP-1 als zentralem Regulator der Sättigung scheint weniger wichtig zu sein als der periphere Effekt von GLP-1 als Stimulator der Insulinsekretion. Nach Meinung der Autoren unterstreichen die Experimente die Wichtigkeit von GLP-1 als physiologischem Regulator des Blutzuckerspiegels. Sie sehen daher Möglichkeiten, GLP-1 in Zukunft therapeutisch in der Diabetesbehandlung einzusetzen.

KOMMENTAR

Anfang der neunziger Jahre wurde es durch neue molekulargenetische Methoden möglich, Tiermodelle mit gezielt ausgeschalteten Genen zu züchten (*Tybulewicz V* et al. Cell 1991; 65: 1153–63; *Nagy A* et al. Proc Natl Acad Sci USA 1993; 90: 8424–8; *Nagy A, Rossant J.* In: Gene targeting: A practical approach. *Joyner AL* (ed.). Oxford, Oxford Univ Press 1993; 147–78; *Wurst W, Joyner AL.* In: Gene targeting: A practical approach. *Joyner AL* (eds.). Oxford, Oxford Univ Press 1993; 33–61). Diese sogenannten »Knockout«-Mutanten sind seither zu wichtigen In-vivo-Untersuchungsmodellen geworden, um zu untersuchen, welche Folgen der Ausfall eines Proteins für den Organismus hat. Da für viele Hormonrezeptoren noch keine hochspezifischen Liganden mit ausschließlich rezeptorantagonistischer Aktivität verfügbar sind, werden zunehmend Tiere mit gezielt ausgeschalteten Rezeptorgenen gezüchtet. Etliche »Knockout«-Konstrukte führen jedoch zu letalen Mutationen, wodurch diese Technik begrenzt wird. In der vorliegenden Arbeit ist es der Arbeitsgruppe von *Drucker* erstmals gelungen, eine Mausmutante zu züchten, die keinen pankreatischen GLP-1-Rezeptor besitzt. Sie konnte ferner zeigen, daß dieser Typ des GLP-1-Rezeptors alle wichtigen biologischen Funktionen von GLP-1 peripher und im ZNS vermittelt. Diese Mausmutante weist nicht nur einen unauffälligen Phänotyp auf, sondern sie entwickelt sich auch normal und ist voll lebensfähig. Sie eignet sich daher ausgezeichnet als Tiermodell für eine komplette GLP-1-Resistenz, die durch ein Fehlen des GLP-1-Rezeptors bedingt ist. Bislang konnte als GLP-1-Rezeptorantagonist lediglich das Exendinfragment Exendin (9–39) (*Göke R* et al. J Biol Chem 1993; 268: 19650–5; *Thorens B* et al. Diabetes 1993; 42: 1678–82; *Kolligs F* et al. Diabetes 1995; 44: 16–9) verwendet werden, das jedoch auch als Antagonist am Rezeptor für Gastric inhibitory polypeptide (GIP) wirkt (*Gremlich S* et al. Diabetes 1995; 44: 1202–8). Da GIP auch die postprandiale Insulinsekretion stimuliert (*Creutzfeldt W.* Diabetologia 1979; 16: 75–85; *Creutzfeldt W, Ebert R.* Diabetologia 1985; 28: 565–73; *Creutzfeldt W, Ebert R.* In: The exocrine pancreas: Biology, pathobiology and disease. *Go VI* et al. (eds.). New York, Raven Press 1986; 333–46), kann durch Experimente mit Exendin (9–39) in vivo keine ausschließliche Aussage über die Wirkung von GLP-1 gemacht werden.

GLP-1 und seine ausgeprägte Stimulation der Insulinsekretion sind seit ungefähr zehn Jahren bekannt (*Schmidt WE* et al. Diabetologia 1985; 28: 704–7; *Mojsov S* et al. J Clin Invest 1987; 79: 619–9; *Kreymann B* et al. Lancet 1987; ii: 1300–4). Von allen gastrointestinalen Hormonen ist GLP-1 der potenteste Stimulator der Insulinsekretion und damit maßgeblich am Inkretineffekt beteiligt (*Orskov C.* Diabetologia 1992; 35: 701–11). Therapiemöglichkeiten des Diabetes mellitus Typ II mit GLP-1 werden seit einigen Jahren intensiv von verschiedenen Arbeitsgruppen untersucht (*Nathan DM* et al. Diabetes Care 1992; 15: 270–6; *Gutniak M* et al. N Engl J Med 1992; 326: 1316–22; *Nauck MA* et al. Diabetologia 1993; 36: 741–4; *Holz GG* et al. Nature 1993; 361: 362–5; *Thorens B, Waeber G.* Diabetes 1993; 42: 1219–25; *Deacon CF* et al. Diabetes 1995; 44: 1126–31; *Ritzel R* et al. Diabetologia 1995; 38: 720–5). Die bei den GLP-1R$^{-/-}$-Mäusen durchgeführten oralen Glukosetoleranztests verdeutlichen eindrucksvoll, welche Auswirkungen das Fehlen der GLP-1-Antwort auf den postprandialen Blutglukose- und Serum-insulinverlauf bei diesen Tieren hat. Überraschend an den zu erwartenden Ergebnissen ist lediglich der ausgeprägte Unterschied im Blutglukoseverlauf bei männlichen und weiblichen GLP-1R$^{-/-}$-Mäusen. Bislang war eine geschlechtsspezifisch unterschiedliche Wirkung von GLP-1 nicht beschrieben worden. Bei zukünftigen klinischen Studien bezüglich Patienten mit Diabetes mellitus Typ II sollte daher auf diesen möglichen Unterschied geachtet werden. Ferner müßte im Tierexperiment geklärt werden, wodurch dieser geschlechtsspezifische Unterschied in der GLP-1 Wirkung vermittelt

wird. Die von *Drucker* und Mitarbeitern gezeigten Verläufe der Insulinkonzentrationen im Serum mit maximaler Stimulation der Insulinsekretion bei den Kontrolltieren 30 Minuten nach oraler Glukosebelastung untermauern frühere Daten, daß GLP-1 vor allem die erste Phase der Insulinsekretion stimuliert (*Weir CG* et al. Diabetes 1989; 38: 338–42; *Orskov C* et al. Diabetes 1993; 42: 658–61). Die Wirkungslosigkeit exogener GLP-1-Gaben bei oralen Glukosetoleranztesten untermauerte bei den transgenen Tieren das völlige Fehlen des GLP-1-Rezeptors oder eines anderen Rezeptors, der biologische GLP-1-Effekte zu vermitteln mag. Leider wurden nicht parallel an den gleichen GLP-1R$^{-/-}$-Mäusen und an den Kontrolltieren neben oralen Glukosetoleranztests auch intravenöse Glukosegaben mit gleicher Blutglukosewirkung vorgenommen. Durch diese Experimente könnte der Inkretineffekt und dessen GLP-1-vermittelter Anteil genau quantifiziert werden.

Aus der Arbeitsgruppe von *Ensinck* und Mitarbeitern gibt es Hinweise, daß GLP-1 nicht nur über eine Stimulation der Insulinsekretion postprandial die Glukosekonzentration im Plasma senkt, sondern auch über insulinunabhängige Mechanismen die Glukoseverwertung stimuliert (*D'Alessio DA* et al. J Clin Invest 1994; 93: 2263–6; *D'Alessio DA* et al. Diabetes 1995; 44: 1433–7; *D'Alessio DA* et al. J Clin Invest 1996; 97: 133–8). Durch GLP-1 wird z. B. auch die Glukagonsekretion gehemmt, woraus niedrigere Blutglukosespiegel resultieren (*Nauck MA* et al. Diabetologia 1993; 36: 741–4; *Ritzel R* et al. Diabetologia 1995; 38: 720–5). *Drucker* und Mitarbeiter postulierten daher, daß die Glukoseverwertung bei den GLP-1R$^{-/-}$-Mäusen, unabhängig von der Art der Zuführung der Glukose, pathologisch sein müßte und beobachteten den Verlauf der Glukosekonzentration nach intraperitonealer Gabe von Glukose. Auch nach intraperitonealer Gabe von Glukose war der Blutzucker bei GLP-1R$^{-/-}$-Mäusen länger und ausgeprägter erhöht als bei Normaltieren. Die Autoren schlossen daraus, daß auch bei Umgehung der enteroinsulinären Achse fehlende GLP-1-Wirkung in einer pathologischen Glukoseverwertung resultiert und daher insulinunabhängige Einflüsse von GLP-1 auf die Glukoseverwertung wichtig sein müßten. Dies ist jedoch nur teilweise richtig. Intraperitoneal applizierte Glukose kann zwar nicht direkt auf physiologischem Weg durch intraluminale Stimulationsmechanismen im Darm GLP-1 freisetzen, es ist jedoch bis jetzt unklar, wie Kohlenhydrate die GLP-1-Freisetzung stimulieren. Die GLP-1-Freisetzung findet vor allem in den L-Zellen des distalen Anteiles des Ileums statt (*Eissele R* et al. Eur J Clin Invest 1992; 22: 283–91), wohin orale Glukose nicht gelangt, weil sie in proximaleren Darmabschnitten bereits resorbiert wurde. Es ist bislang nicht völlig ausgeschlossen, daß neben nervaler Stimulation durch Dehnungsreize in proximalen Abschnitten des Gastrointestinaltraktes auch der Glukoseanstieg im Portalvenenblut zur vermehrten Freisetzung von GLP-1 führt. Durch intraperitoneale Gabe von Glukose wird zumindest im Portalkreislauf kurzfristig auch eine erhöhte Glukosekonzentration anfluten, die der von oral gegebener Glukose entspricht. Lediglich der hemmende Effekt von GLP-1 auf die Magenentleerung (*Willms B* et al. J Clin Endocrinol Metab 1996; 81: 327–32) wird bei intraperitonealer Glukosegabe im Vergleich zu oraler Gabe umgangen. Ob extrapankreatische Effekte von GLP-1 (z. B. Stimulation der Glukoseaufnahme in Skelettmuskel oder Fettgewebe, Stimulierung der Glykogensynthese in der Leber) (*Egan JM* et al. Endocrinology 1994; 135: 2070–5; *Villanueva-Penacarillo ML* et al. Diabetologia 1994; 37: 1163–6; *D'Alessio DA* et al. J Clin Invest 1994; 93: 2263–6; *D'Alessio DA* et al. Diabetes 1995; 44: 1433–7) bei der Glukoseverwertung eine Rolle spielen, läßt sich mit dem von *Drucker* und Mitarbeitern gewählten Ansatz der intraperitonealen Glukosegabe weder klären noch quantifizieren. Hierzu wären Untersuchungen in vitro an o. g. Geweben von mutanten Mäusen ohne GLP-1-Rezeptor und von normalen Kontrollen nötig. Da sich die GLP-1R$^{-/-}$-Mäuse leicht vermehren lassen, sind diese Untersuchungen in Zukunft problemlos durchführbar.

Die kürzlich publizierten, aufsehenerregenden Befunde der Arbeitsgruppe von *Bloom*, die GLP-1 eine wichtige Rolle als zentralem Mediator der Sättigung zubilligen (*Turton MD* et al. Nature 1996; 379: 69–72), werden durch die Untersuchungen an GLP-1R$^{-/-}$-Mäusen erheblich relativiert. Zum einen weisen diese Mäuse auch über einen langen Zeitraum ein normales Körpergewicht auf, zum anderen ist ihr Freßverhalten nach einer Nüchternperiode unauffällig. Icv-Gaben von GLP-1 waren bei diesen Tieren wirkungslos, was beweist, daß im ZNS der gleiche »pankreatische« GLP-1-Rezeptor vorliegt wie in anderen Organen. Autoradiographien mit ^{125}J-markiertem GLP-1 an Hirnschnitten von GLP-1R$^{-/-}$-Mäusen zeigten, daß keine anderen GLP-1-Bindungsstellen im ZNS zusätzlich vorliegen. Durch diese Befunde wird offensichtlich, daß eine Regulation des Freßverhaltens und des Körpergewichtes keineswegs zum größten Teil durch zentrales GLP-1 stattfindet. Es ist seit langem bekannt, daß viele Neuropeptide eine Rolle bei der zentralen Regulation der Nahrungsaufnahme spielen (*Morley JE* et al. Endocr Rev 1987; 8: 256–87; *Lee MC* et al. Neurosci Behav Rev 1994; 18: 313–23). So sind Cholezystokinin (CCK) (*Moran TH* et al. Am J Physiol 1993; 265: G219–23) und Bombesin an der Vermittlung der Sättigung beteiligt, wohingegen Neuropeptid Y (NPY) und Galanin das Freßverhalten stimulieren (*Schick RR* et al. Brain Res 1991; 552: 232–9; *Schick RR* et al. Am J Physiol 1993; 264: R355–61). Über die zentralnervöse Interaktion dieser Peptide ist bislang wenig bekannt. Für GLP-1 konnte jedoch gezeigt werden, daß es zentral über NPY-unabhängige Mechanismen wirkt (*Turton MD* et al. Nature 1996; 379: 69–72). Ferner scheint das Produkt des ob-Gens, Leptin, eine wichtige Rolle bei der zentralen Regulation der Sättigung zu spielen (*Pelleymounter MA* et al. Science 1995; 269: 540–3; *Halass JL* et al. Science 1995; 269: 543–6; *Campfield LA* et al. Science 1995; 269: 546–9). Bei Mäusen mit ausgeschaltetem NPY-Gen, die im übrigen wie die GLP-1R$^{-/-}$-Mäuse nicht adipös waren und normales Freßverhalten zeigten, ließ sich eine erhöhte Leptinsensitivität beobachten (*Erickson JC* et al. Nature 1996; 381: 415–8). Für die Mäuse mit ausgeschaltetem GLP-1-Rezeptorgen ist die Leptinempfindlichkeit noch nicht untersucht. Die

sicherlich komplexe Wirkung und Interaktion von GLP-1 mit anderen Neuromodulatoren im ZNS muß weiter geklärt werden, hierzu wären weitere Untersuchungen an GLP-1R$^{-/-}$-Mäusen sinnvoll.

FAZIT

Die vorliegende Arbeit liefert einen weiteren Beweis für die Wichtigkeit von GLP-1 als Inkretinhormon. Die bei den GLP-1R$^{-/-}$-Mäusen pathologischen Verläufe der Glukosekonzentrationen nach oraler oder intraperitonealer Glukosegabe unterstreichen den Einfluß von GLP-1 bei der Kontrolle der Glukosehomöostase und liefern Argumente dafür, daß GLP-1 möglicherweise Potential für die Therapie des Diabetes mellitus Typ II besitzt. Neben diesen wichtigen Ergebnissen zur Physiologie von GLP-1 zeigt die vorliegende Arbeit erneut, daß »Knockout«-Mäuse bei geeignetem experimentellem Ansatz brauchbare Versuchsmodelle sind, um gezielt die physiologischen Effekte des Fehlens eines Proteins zu untersuchen.